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VARIABILITY OF PATHOGENIC MICROORGANISMS

- M.S. Zakharova

The problem of the variability of pathogenic microorganisms implies a large number of theoretical and practical questions. Soviet microbiologists study this problem in all its aspects. They are seeking a solution to theoretical questions important to general biology. They also seek to use the results acquired in a practical way against infectious diseases as it concerns diagnostic bacteriology, specific prophylaxis and treatment.

~~In my report~~ ^{The} I consider only two questions which have been studied on a large scale and with success in the laboratories of our institute, notably the questions of formation of bacteria resistant to therapeutic substances, controlled variability of microorganisms and the formation of species.

Lately the problem of medicinal resistance of microbes is drawing attention particularly of microbiologists as well as of clinicians.

The capacity of bacteria to acquire resistance against antiseptic substances was shown for the first time by G. Kossjakov in 1887. In his experiments *Bacillus anthrax*, *Bacillus subtilis* and other microbes cultivated on media containing increasing concentrations of boric acid, mercuric chloride and borax became more resistant to these substances.

Twenty years later Erlich described the same phenomenon with arsenical preparations in the treatment of mice infected by tripanosomes or spirochetes.

A series of researches has shown lately, during the period of the development of chemotherapy of bacterial infections that it is possible to obtain experimentally forms of different bacterial species resistant to sulfamides and antibiotics. Other research has shown that it is also possible to isolate these forms of the disease treated with these preparations. One cannot explain the mechanism of the appearance of resistance, since the intimate mechanism of antibacterial action of antibiotics is not yet completely understood.

Nevertheless the correct manner of conditioning the formation of resistant bacteria, from the point of view of general biology, is of great interest not only theoretically but also from the point of view of organizing correct measures for anticipating this phenomenon.

- In this sense Professor Troitski, Perchina and others have carried out very interesting research in our institute. These studies have shown that medical resistance of bacteria represents a particular case of the general biological law - capacity of cells to accustom themselves and adapt to unfavorable factors in the culture medium.

In a bacterial population of *B. coli* or staphylococci without a bacterial cell resistant to penicillin or streptomycin respectively, the authors obtained bacterial forms resistant to the antibiotics through a limited number of passages on medium containing the antibiotic. This research shows that the creation of bacterial forms resistant to antibiotics is equivalent to the influence of the exterior environment which leads to modification of the metabolism, becoming hereditarily fixed. They contradict the beliefs of some scholars concerning the origin of bacterial forms resistant to therapeutic substances by means of spontaneous mutation.

In order to study the mechanism of lysis of microbes under the action of penicillin, as well as to observe the dynamics of the formation of resistant bacteria, the soviet authors above-mentioned have made a microphotograph of the preparations made.

The photographs have revealed the mechanism of lysis of microbes under the influence of penicillin. It is apparent that the process develops in the following manner: the elongated microbes form a thickening in the middle of the cell which gives them a fusiform shape, during the enlargement may appear nonconformity between the intracellular pressure of cytoplasm and the capacity of the cell membrane to stretch, the membrane thins (first at the point of enlargement of the cell) springs out again forming a spherical swelling which is filled by the liquid content of the bacterial cell and is torn at the moment of further increase of intracellular pressure. The content of the cell bursts from the tear in the membrane and there remains of the bacterial cell only the stroma, having the appearance of a pale shadow which feebly refracts the light and conserves the contour of the destroyed cell.

Figure 1 reproduces the isolated outlines of the part of the film where lysis of the *B. coli* is represented under action of penicillin. Figure 2 represents the outlines of film showing the process of budding of the maternal cell, the daughter cells which begin to be divided normally and give the generation of forms of *B. coli* resistant to penicillin.

We have observed the same conformity to law in another morphological manifestation of the death of the microbial cell during the action of other antibiotics and sulfamidine preparations on microbes of the intestinal group. Thus, from a bacterial colony of *B. coli* or of staphylococci not containing bacterial cells resistant to penicillin or streptomycin it is possible to obtain

forms of the microbe resistant to a high concentration of the above-mentioned antibiotics by means of a minimum number of passages on the media containing the antibiotics. As a consequence, contrary to the conceptions of researchers who consider that microbes with therapeutic resistance are products of spontaneous mutation, it has been proved that antibiotic resistance is an acquired characteristic in bacterial cells under the action of sub-bactericidal doses of the antibiotic.

Bacteria under the influence of the antibiotic become not only more resistant toward this substance, but this newly acquired characteristic is transmitted from one generation to the other as hereditary, thus there may appear a stock of bacteria resistant to penicillin.

This research has also made clear the morphological manifestations of the mechanism of the formation of bacteria resistant to penicillin. It is established, by means of photo and observation, that the bacterial cell sensible to penicillin, reacting by modification of its initial form to an elongated fusiform and by retarding of its segmentation under action of penicillin, detaches a daughter cell resistant to penicillin, which gives in the course of later segmentation, a generation of bacterial forms resistant to penicillin. Thus, the data of morphological research represents a demonstrative example of the heredity of acquired characteristics.

Study of ~~xxxxxxx~~ controlled variability of microorganisms, learning the laws of mechanisms determining this phenomenon have certainly a great importance from the theoretical and practical point of view. On the solution of this problem depends the solution of theoretical questions in general biology concerning heredity and variability, evolution of microorganisms, genetic possibilities, and reciprocal relations of microorganisms, information on species and processes of formation of species in the world of microorganisms.

The problem of controlled modification of hereditary properties of microorganisms and obtaining, thanks to that, of variants which may be used in the interest of humanity and in particular in the fight against infectious diseases, is not new to the microbiologist. This problem has concerned researchers during the whole history of this science.

Pasteur, Metchnikov, Tsenkovski, Vinogradski, Onelianski, Gamaleia and others not only expressed the idea of controlled modification in the nature of microbes, but performed determinative experiments. Their research elaborates certain principles of preventive vaccination and concerns obtaining vaccines which have not lost their strength in the bearer (vaccine against rabies, anthrax etc.)

Lately soviet microbiologists have done research which gives the possibility of clearing up certain laws and causes determining the processes of variability and the formation of species. Their works enable us to draw certain conclusions of a general nature concerning this problem.

Permit me to dwell on some problems which have great importance from my point of view.

A series of experiments having great importance from the point of view of general biology on the study of modification and transformation from one species of microbe into another, was performed by Zilber (1928), Krassilnikov (1941-1945), Gamaleia and Gratcheva (1946), Lenskaja and other authors.

Cultivating *Proteus vulgaris* in a sack of collodium introduced into the abdominal cavity of a guinea pig infected with the rickettsia of exanthematic typhus, Zilber obtained a culture with the serological characteristics of *Provatzei* which agglutinated for the serum of a person tainted with exanthematic typhus, also to proteus 19. This stock *B. proteus* keeps its modification of above-mentioned properties for 19 years.

Krassilnikov has stated that leguminous microbes of melilot, vetch, alfalfa and others provoke further the development of tubercles on the roots of the alfalfa. Several strains keep these properties for 5 months.

Cultivating *B. coli* in Tirode liquid deprived of nitrogen, containing killed microbial cells of *Salmonella* Breslau, Gratcheva who did his research under the direction of N.E. Gamaleia observed a successive transformation of *B. coli* into paratyphoid bacillus of the Breslau type.

These works began to be developed more intensely in 1948 after the known session of the Academy of Agricultural Sciences (Lenin). Our laboratory (Zakharova, Sviridova and others), the laboratory of Professor Timakov (Koudlai, Semtcheva and others) performed research to the end of elucidating the laws of formation of bacterial species of the intestinal-typhoid group and in the hope of finding methods of guided modification of microorganisms in new forms useful to man.

Conforming to the studies of U.V. Mitchourine, T.D. Lysenko and their collaborators who have proved the possibility of formation of new species and forms of plants, the research allows us to consider this law proper also to microorganisms.

Using their research on the essential principles of Mitchourine's biology, according to which variations of the animal body occur due to deviation from the norm for the type, of assimilation and dissimulation, and due to modification of the type of metabolism, the researchers created conditions of nutrition for the microbes by which natural cells are distinguished from laboratory cells.

As plastic substance introduced artificially in the process of metabolism of controlled bacteria, they used microbial bodies killed by heat, as well as nucleoproteides, complete antigens, desoxyribonucleic acid and autoclaved filtrates of bacteria of "the controlled species" which were added to the synthetic medium (Tirode liquid), deprived of other sources of nitrogen.

Cultivating *B. coli* on the product of disintegration of Broslau's paratyphoid bacteria, Chotmuller's paratyphoid, and abdominal typhus, they succeeded in obtaining stable variants with fixed hereditary characteristics, peculiar to these bacterial species, and on the other hand, during cultivation on products of disintegration of *B. coli* the paratyphoid microbes acquired characteristics of *B. coli*. On cultures with characteristics of the controlled species in these conditions, they obtained new forms of microorganisms named "alkali formers". Ordinarily they appeared in the 3-4 generation, if the cultivation was effected under aerated conditions the alkali formers were rarely manifest. They resemble almost entirely the bacterial species known in microbiology as *F. alcaligenes*, which certain authors relate to another genera of bacteria.

The first modifications of the properties of microorganisms at the time of these experiments appeared at the 4-6 passage. At the 10-17 passage the microbe undergoing modification acquired the majority of properties and characteristics "of the controlled culture". But at the end of this period, the characteristics newly acquired are not yet fixed in a stable manner. In this period, reversion - return to the initial form - can be accomplished easily. The newly acquired properties are fixed in a stable manner at the end of different terms, which depends on the specific properties of the culture and the strains which undergo this experience as well as the conditions of cultivation of the microbe.

After the formation of a new species of microorganism a profound modification is produced in the character of the processes of assimilation and dissimilation - the hereditary properties of the microorganisms "are shaken". In this period quantitative modifications are produced, which accumulate in successive generations of microbes and finally they are succeeded in an irregular manner (in leaps) by qualitative modifications which are hereditarily fixed, conforming to conditions in the changed life of the microbes.

This conception is confirmed by the modification of the biochemical properties and especially by serological properties of the microbes. These properties reflect in the most exact manner modifications of the processes of metabolism and biosynthesis of the microbial cell. It has been established from these experiences that microorganisms of the intestinal group modified in a controlled manner are agglutinated during the first passages (up to 12-14) by the homological serum, by the heterological serum of the controlled culture, and by the serum of "alkali formers".

The cultures obtained during this period have great serological polyagglutinability; they have different properties from the microbes used for this experiment. The characteristics revealed prove profound modifications, which are produced in the metabolism as well as the biochemical structure of the bacteria, but the stable hereditary fixation of these modifications is produced much later.

As can be seen on figures 3 and 4 the processes of agglutination already have another characteristic at the 15-16 passage. At this time the "antiserum" agglutinability of the controlled culture becomes more intense, but the titre of agglutination with the homological antiserum of the culture undergoing modification, diminishes rapidly.

During this period, as a rule, the microbial species newly formed received all the biochemical properties of the controlled culture. One observes something equal to a "crisis", a jump, after which the properties and characteristics acquired are hereditarily fixed. The duration of the process of formation of species of different microbes is not identical. Thus from cultivation of *B. coli* in medium containing bacteria of Breslau paratyphoid killed with heat, the process of formation of species terminated at the 15-17 passage. If one uses, under analogous conditions, a combination of *B. coli* with bacteria of abdominal typhus, it terminates at the 25-35 passage, since with bacteria of Flexner dysentery this process is longer. Consequently the transformation from one species of microbe into another occurs as a struggle between the new and the old, when the old has a tendency to reconstitute itself under the conditions of the new which is formed. This process is extraordinarily complicated.

In the process of formation of a species there is revealed in a precise manner the antagonistic relation between the old and the new species which is born. The new species shows itself more capable of adapting to new qualitative conditions of existence than the old. So to speak, the new supplants the old. The new successfully coincides with the old, becomes antagonistic toward it. In figures 5 and 7 one sees the compatible growth of *B. coli* and "alkali formers", artificially produced. The last "bite" so to speak, destroys the culture of *B. coli*. Figure 7 illustrates a greater viability of the species which is being born in comparison with the old. One sees on this figure the correlation of the number of colonies of *B. coli* and of the species formed. The figure shows that with the augmentation of number of passages, the quantity of colonies with modified characteristics is increased, which supplants the old colonies less adapted to new conditions of life.

As different as may be the factors on which depend the modifications of hereditary properties of microorganisms, the essential cause is modification of the processes of assimilation

and dissimilation, the modification of the character of metabolism. Our task is to elucidate and study in a serious way and in all aspects, the mechanism of metabolism which occur in process of modification of microbial properties and which condition the law of formation of species linked with the original conditions of life. All this will give us the possibility of controlling modifications of the properties of microbes in each case according to the wish and desire of the researcher.

The happy solution of these questions will enable us to solve with success the problem of specific prophylaxis of several infectious diseases. Thus the exclusively honorary and noble task of liquidation of all epidemic diseases, which is presented to soviet research, will receive a real basis for accomplishment.